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Filing Date	April 15, 2003
First Named Inventor	Daniel DUPRET et al.
Art Unit	1627
Examiner Name	Steven C/ Tizio
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Total Number of Pages in This Submission: 15

ENCLOSURES (*Check all that apply*)

- [ ] Fee Transmittal Form
- [ ] Fee Attached
- [X] Amendment/Reply
- [ ] After Final
- [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
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- [ ] Information Disclosure Statement
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Remarks

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of :	)	
	)	
Daniel DUPRET *et al.*	)	Examiner: Tizio, S.
	)	
Serial No.: 09/723,316	)	Group Art Unit: 1627
	)	
Filed: November 28, 2000	)	

For: METHOD FOR OBTAINING *IN VITRO* RECOMBINED POLYNUCLEOTIDE SEQUENCES, SEQUENCE BANKS AND RESULTING SEQUENCES

Commissioner of Patents
Washington, D.C. 20231

AMENDMENT IN RESPONSE TO JANUARY 15, 2003 OFFICE ACTION

Sir:

IN THE SPECIFICATION:

Please add the following:

At page 1, beginning at line 5, please insert the following paragraph:

-- CROSS-REFERENCE TO RELATED APPLICATIONS

This application is entitled to the benefit of PCT Application No. PCT/FR99/01973, filed August 11, 1999; and French Patent Application No. FR98/10338, filed August 12, 1998. --

On a separate sheet, please add the following abstract:

- - ABSTRACT OF THE DISCLOSURE

Ligation-mediated method of recombining polynucleotides *in vitro*. Polynucleotides from a library are fragmented and the fragments are hybridized to an assembly template. The hybridized fragments are iteratively re-hybridized and ligated until the ends of the hybridized fragments are adjacent to the ends of other hybridized fragments on the assembly template. A final ligation produces recombined polynucleotides. - -

Please replace the paragraphs indicated below with the following paragraphs. Marked-up versions showing the changes are attached.

The paragraph at p. 11, 3rd para., line 26 to p. 12, line 6:

The invention has for its object the generation of polynucleotide sequences liable to have advantageous properties as compared to the corresponding properties of reference sequences. The recombined polynucleotide sequences obtained at step (d) and possibly cloned are screened by any appropriate means in order to select the recombined polynucleotide sequences or the clones having advantageous properties as compared to the corresponding properties of the reference sequences. By advantageous property is understood to be, for example, the thermostability of an enzyme or its ability to function under conditions of pH or of temperature or of saline concentration more adapted to an enzymatic process than the control proteins usually used for said process. For example, such a process can be an industrial process to breakdown textile fibers or bleaching paper pulps or producing flavors in the dairy industry, the processes of biocatalysis for the synthesis by an enzymatic pathway of new therapeutic molecules, etc.

The paragraph at p. 17, 2nd para., lines 11-18:

The product of the five PCR was mixed and loaded on a 1% TBEagarose gel after migration and staining of the gel with ethidium bromide, the band at 2651 bp, corresponding to the *ponB* gene amplification product surrounded by two fragments of 26 bp and 90 bp respectively, was visualized by trans-illumination under ultraviolet, and cut out with a scalpel in order to be purified with the QIAquick system (QIAGEN). All the DNA thus purified was eluted in 120 µl of buffer T. The concentration of this DNA was approximately 100 ng/µl as measured by its absorbance at 260 nm.

The paragraph at p. 18, 4th para., lines 21-26:

50 µl of each of the ten PCR were mixed and loaded on a 1% TBE agarose gel. After migration and staining with ethidium bromide, the band at 2572 bp, corresponding to the amplification product of the genes of the ten mutants, was cut out with a scalpel and purified with the QIAquick system (QIAGEN). All the DNA thus purified was eluted in 120 µl of buffer T. The concentration of this DNA was approximately 100 ng/µl according to its absorbance at 260 nm.

The paragraph at p. 21, 2nd para., lines 8-12:

The PCR amplification products of the RLR 1, 2 and 3 reactions were purified with the WIZARD PCR PREPS system (PROMEGA) and eluted in 45 µl of buffer T. 6 µl of each purified PCR were incubated 1 hour at 37 °C in a mixture containing 3 µl of restriction buffer C, 3 µl of BSA (1 mg/ml), 20 U of the *Eco* RI enzyme, 10 U of the *Nco* I enzyme and 15 µl of water.

The paragraph at p. 21, 4th para., lines 20-23:

The linearized vectors as well as the digested PCR were purified on a TBE 1% agarose gel with the QIAquick system (QIAGEN). Each vector or each digested PCR was eluted in 30 µl of buffer T.

IN THE CLAIMS:

Please cancel claims 1-39.

Please add the following new claims:

40. A ligation-mediated *in vitro* method of recombining polynucleotides from a polynucleotide library, comprising:

fragmenting polynucleotides from the library,

hybridizing the fragments to an assembly template,

ligating the hybridized fragments,

optionally denaturing the hybridized fragments from the template,

repeating the hybridizing step, before or after the ligating step or the denaturing step, as necessary until ends of the hybridized fragments are substantially adjacent to each other on the assembly template, and

ligating the adjacent ends to form a recombined polynucleotide.

41. The method of claim 40, wherein any polymerase used in the method is used only to amplify the recombined polynucleotide, to amplify polynucleotides from the library, or to amplify the fragments before the first hybridizing step.

42. The method of claim 40, further comprising adding assembly templates before completion of the hybridizing step(s).

43. The method of claim 40, wherein the polynucleotides from the library are double-stranded and these double-stranded polynucleotides or fragments thereof are denatured before the hybridizing step(s).

44. The method of claim 40, further comprising selecting the recombined polynucleotide that possesses advantageous properties.

45. A ligation-mediated *in vitro* method of recombining polynucleotides from a polynucleotide library, comprising:

fragmenting polynucleotides from the library,

hybridizing the fragments to an assembly template,

ligating the hybridized fragments,

denaturing the hybridized fragments from the template,

repeating the hybridizing step multiple times, before or after the ligating step or the denaturing step, until ends of the hybridized fragments become substantially adjacent to each other on the assembly template, and

ligating the adjacent ends to form a recombined polynucleotide.

46. The method of claim 45, wherein any polymerase used in the method is used only to amplify the recombined polynucleotide, to amplify polynucleotides from the library, or to amplify the fragments before the first hybridizing step.

47. The method of claim 45, further comprising adding assembly templates before completion of the hybridizing steps.

48. The method of claim 45, wherein the polynucleotides from the library are double-stranded and these double-stranded polynucleotides or fragments thereof are denatured before the first hybridizing step.

49. The method of claim 45, further comprising selecting the recombined polynucleotide that possesses advantageous properties.

50. A ligation-mediated *in vitro* method of recombining polynucleotides from a polynucleotide library, comprising:

fragmenting polynucleotides from the library,

hybridizing the fragments to an assembly template,

ligating the hybridized fragments,

optionally denaturing the hybridized fragments from the assembly template, and

repeating the hybridizing step, before or after the ligating step or the denaturing step, as necessary to form a recombined polynucleotide,

wherein any polymerase used in the method is used only to amplify the recombined polynucleotide, to amplify polynucleotides from the library, or to amplify the fragments before the first hybridizing step.

51. The method of claim 50, wherein, before the last ligating step, the ends of the hybridized fragments are substantially adjacent to each other on the assembly template.

52. The method of claim 50, further comprising adding assembly templates before completion of the hybridizing step(s).

53. The method of claim 50, wherein the polynucleotides from the library are double-stranded and these double-stranded polynucleotides or fragments thereof are denatured before the hybridizing step(s).

54. The method of claim 50, further comprising selecting the recombined polynucleotide that possesses advantageous properties.

55. The method of claim 44, further comprising amplifying the recombined polynucleotide before the selecting step.

56. The method of claim 44, further comprising separating the recombined polynucleotide from the assembly template before the selecting step.

57. The method of claim 56, further comprising cloning the recombined polynucleotide after the separating step and before the selecting step.

58. The method of claim 40, wherein the assembly template comprises oligonucleotides that are complementary to the 3' ends of some of the fragments and to the 5' ends of some of the other fragments.

59. The method of claim 40, wherein substantial portions of the library polynucleotides are homologous to each other or complementary to portions of the assembly template.

60. The method of claim 40, wherein substantial portions of the hybridized fragments are complementary to portions of the assembly template that are substantially adjacent to each other.

61. The method of claim 40, wherein the hybridizing and ligating steps are carried out simultaneously.

62. The method of claim 40, wherein the fragmenting is random.

63. The method of claim 62, wherein the random fragmenting comprises treatment with DNAse I and wherein the library comprises partially heterologous double-stranded polynucleotides.

64. The method of claim 40, wherein a researcher using the method controls the fragmenting and chooses the degree of recombination, the points of recombination, or both.

65. The method of claim 64, wherein the fragmenting comprises hydrolyzing the polynucleotides from the library with restriction enzymes.

66. The method of claim 65, wherein the hydrolyzing is performed with several different restriction enzymes or with restriction enzymes that have a plurality of cutting sites on the polynucleotides from the library.

67. The method of claim 65, wherein the hydrolyzing comprises separately hydrolyzing polynucleotides from at least two distinct polynucleotide libraries by subjecting the distinct libraries to different restriction enzymes.

68. The method of claim 40, further comprising adding enzymes that recognize and cut non-hybridized ends of the hybridized fragments in a specific manner when the non-hybridized ends overlap other hybridized fragments on the same assembly template.

69. The method of claim 68, wherein the enzyme is a flap endonuclease.

70. The method of claim 68, wherein the ligating step(s) utilizes a ligase that is thermostable and active at high temperature.

71. The method of claim 70, wherein the enzymes that recognize and cut the non-hybridized ends have the same thermostability and high temperature activity as the ligase.

72. The method of claim 40, wherein some of the fragments serve as the assembly template for some of the other fragments.

73. The method of claim 40, wherein the library consists essentially of polynucleotides obtained by successive steps of directed mutagenesis, by error prone PCR, by random chemical mutagenesis, by *in vivo* random mutagenesis from a single gene, or by recombining genes of close or distinct families within the same or different species.

74. The method of claim 40, wherein the library comprises synthetic polynucleotides.

75. The method of claim 40, wherein, before the first hybridizing step, the template is double stranded.

76. A vector comprising the selected recombined polynucleotide of claim 44, 49 or 54.

77. A cellular host transformed by the selected recombined polynucleotide of claim 44, 49 or 54.

78. A protein encoded by the selected recombined polynucleotide of claim 44, 49 or 54.

79. A library formed from the recombined polynucleotide of claim 40, 45 or 50.

80. A library formed from the selected recombined polynucleotide of claim 44, 49 or 54.

REMARKS

Applicant respectfully requests entry of the foregoing and reconsideration of the application in light of the following remarks.

Claims 1-39 have been canceled. Claims 40-80 have been added. Claims 40-80 add no new matter. Although a few of the terms and phrases in claims 40-80 are not literally recited in the specification or in the previous claims, those terms and phrases are inherently or implicitly present in the specification and in the previous claims.[1]

Claims 40-80 have not been added to overcome the prior art rejections. The main reason for adding claims 40-80 was to conform to standard English and to enhance readability, clarity and brevity.[2]

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[1] *See generally Ex parte Holt*, 19 USPQ2d 1211, 1213 (PBAI 1991) (It is well established that the invention claimed need not be described *ipsis verbis* in order to satisfy the disclosure requirement of §112.); *In re Smythe*, 480 F. 2d 1376, 178 USPQ 279 (CCPA 1973) (By disclosing in a patent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter.); *Behr v. Talbot*, 27 USPQ2d 1401, 1407 (BPAI 1992) (It is not necessary for the application to reveal a conscious appreciation on the part of the applicants of the significance of the limitation in question.); *Ex parte Parks*, 30 USPQ2d 1234, 1236 (BPAI 1994) (Clearly, the observation of a lack of literal support does not, in and of itself, establish a *prima facie* case for lack of adequate descriptive support....In the situation before us, it cannot be said that the originally-filed disclosure would not have conveyed to one having ordinary skill in the art that appellants had possession of the *concept* of conducting the decomposition step generating nitric acid in the absence of a catalyst.); *Ex Parte Yamaguchi*, 6 USPQ2d 1805, 1807 (PTO Bd App & Int 1987) (It is well settled in patent law that a compound and all of its properties are inseparable. Accordingly, where a compound is disclosed in such a manner as to comply with §112, 1st para., the later addition of symbols by which the compounds can be identified, classified and compared (such as x-ray diffraction spectra, graphic formula, chemical nomenclature) do not define a separate invention.); *In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970) ("[W]here no explicit description of a generic invention is to be found in the specification mention of representative compounds may provide an implicit description upon which to base generic claim language."); *In re Alton*, 37 USPQ2d 1578, 1581 (Fed. Cir. 1996) (If the specification contains a description of the claimed invention, albeit not in *ipsis verbis*, then the examiner or Board, in order to meet the burden of proof, must provide reasons why one of ordinary skill in the art would not consider the description sufficient.). *See also* MPEP 2163.07(a) Inherent Function, Theory, or Advantage.

[2] For example, new independent claims 40, 45 and 50 recite a denaturation step after the ligation step, whereas previous claim 1 recited a denaturation step before the first hybridization step. This does not mean Claims 40, 45 and 50 exclude denaturation before the first hybridization step. Claims 40, 45 and 50 encompass the use of single-stranded and double-stranded fragments. Since

(continued...)

Please note also that some terms in the previous claims were either explicitly in the plural form (*e.g.*, "assembly templates") or explicitly in the singular and plural form (*e.g.*, "assembly template or templates"). Now the terms appear in the implicit singular/plural form (*i.e.*, "an assembly template"). Thus, these terms encompass both the singular and the plural (*i.e.*, "an assembly template" encompasses one or more assembly templates.)

Objections to Specification

The amendments above satisfy the objections in Paragraphs 1-3 of the Office Action.

With regard to Paragraph 4 of the Office Action, Applicant thanks the Examiner for suggesting a preferred layout for the specification. However, Applicants prefer to retain the current layout.

Objections to Claims

The objectionable claims have been canceled. New claims 40-74 do not have the objectionable features recited in Paragraphs 5-7 of the Office Action.

Claim Rejections Under 35 USC 112

The claims rejected under Section 112 have been canceled. New claims 40-74 do not have the offending features recited in Paragraph 8(A)-(I) of the Office Action.

Claim Rejections Under 35 USC 102(e)

Previous claims 1-13, 15, 17-29 and 31-39 are rejected under 35 USC. 102 (e) as anticipated by US Pat No. 6,117,679 to Stemmer. However, Stemmer's method is polymerase-mediated.

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denaturation is always necessary when double-stranded fragments are to be hybridized to the template, the initial denaturation need not be explicitly recited. On the other hand, claims 40, 45 and 50 now recite denaturation after the first hybridization. The reason is to clarify that denaturation may occur prior to any further rounds of hybridization. In other words, denaturation after the first hybridization is not necessarily inherent in all embodiments of the invention, so it is recited in he claims.

Stemmer does not disclose or teach the ligation-mediated method of Applicant's previous claims or of new claims 40-74.

As stated in Applicant's specification, "The key step of the process of the invention is the step of ligation on an assembly template" (page 5, lines 2-3). The present invention differs from the prior art because the present invention does not, among other things, rely on polymerase to fill in large gaps between fragments hybridized on a template. Rather, the invention repeats hybridization as necessary until fragments hybridize in the gaps.[3] In other words, the invention does not hybridize a small number of fragments to a template and then polymerize the ends of the hybridized fragments toward each other until they are within ligatable distances. Instead, the invention iteratively cycles through rounds of hybridization so that more fragments hybridize to the template and so that the hybridized fragments grow in length (without polymerase extension), until a sufficient number fragments of sufficient lengths are within ligatable distances of each other.[4]

That Stemmer's method relies on polymerase extension is evident in the following passages:

-Col. 22, lines 24-26: "In shuffling, however, the number of the polymerase start sites and the number of molecules remains essentially the same."

-Col. 24, lines 37-38: "The annealed nucleic acid fragments are next incubated in the presence of a nucleic acid polymerase and dNTPs".

-Col. 24, lines 53-56: "The cycle of denaturation, renaturation and incubation in the presence of polymerase can be referred to as shuffling or reassembly of the nucleic acid."

Also conspicuous is the absence of relevant disclosure in Stemmer. Stemmer does not disclose features necessary for or consistent with Applicant's ligation-mediated method, such as the following:

-Ends of fragments adjacently hybridize on the assembly template.

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[3] Repeating the hybridizing step often also entails repeating the ligating and denaturing step immediately after each repetition of the hybridizing step. However, it is possible to repeat the hybridizing step multiple times before any ligating or denaturing.

[4] In typical embodiments of the invention, multiple rounds of hybridization are usually necessary. Under certain conditions, however, a sufficient number of fragments of sufficient lengths can hybridize within ligatable distances of each other. In Claims 40 and 50, the phrase "as necessary" allows for this unusual scenario. Claim 45, however, does not encompass this unusual scenario because it lacks "as necessary" and it always requires "multiple" rounds of hybridization.

-Repeating the hybridizing and ligating steps with the ligated and non-ligated fragments.

-Carrying out the hybridization and ligation steps simultaneously.

-Polynucleotides of the library share zones of homology among each other or with the assembly template. In other words, portions of the library polynucleotides are homologous to each other or complementary to the assembly template. This facilitates adjacent hybridization of the fragment ends on the assembly template.

Claim Rejections Under 35 USC 103(a)

Previous claims 14 and 16 are rejected under 35 USC. 103(a) as obvious over Stemmer in view of US6,251,649 to Matsui et al. The Office Action relies on Matsui et al for disclosure of flap endonuclease that is thermoresistant and active at high temperature. Because Stemmer fails to disclose or suggest the method of any of Applicant's previous or new claims, the combination of Stemmer and Matsui et al also fails to suggest the method of any of Applicant's previous or new claims.

Applicant respectfully requests entry of the amendments and the new claims and issuance of a Notice of Allowance.

Please charge any shortage in fees due in connection with the filing of this Amendment to Deposit Account No. 50-0206, and please credit any excess fees to such deposit account.

Respectfully submitted,

HUNTON & WILLIAMS

Dated: 4-15-03 By: _____

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Marked-Up Version of Amendments to Specification Paragraphs

Paragraph at page 11, third paragraph, line 26 to page 12, line 6:

The invention has for its object the generation of polynucleotide sequences liable to have advantageous properties as compared to the corresponding properties of reference sequences. The recombined polynucleotide sequences obtained at step (d) and possibly cloned are screened by any appropriate means in order to select the recombined polynucleotide sequences or the clones having advantageous properties as compared to the corresponding properties of the reference sequences. By advantageous property is understood to be, for example, the thermostability of an enzyme or its [abilityto] ability to function under conditions of pH or of temperature or of saline concentration more adapted to an enzymatic process than the control proteins usually used for said process. [Forexample] For example , such a process can be an industrial process to breakdown textile fibers or bleaching paper pulps or producing flavors in the dairy industry, the processes of biocatalysis for the synthesis by an enzymatic pathway of new therapeutic molecules, etc.

Paragraph at page 17, 2nd paragraph, lines 11-18:

The product of the five PCR was mixed and [loadedon] loaded on a 1% TBEagarose [gelAfter] gel after migration and staining of the gel with ethidium bromide, the band at 2651 bp, corresponding to the *ponB* gene amplification product surrounded by two fragments of 26 bp and 90 bp respectively, was visualized by trans-illumination under ultraviolet, and cut out with a scalpel in order to be purified with the [QUIAquick] QIAquick system (QIAGEN). [Allthe] All the DNA thus purified was eluted in 120 µl of buffer T. The concentration of this DNA was [approximatively] approximately 100 ng/µl as measured by its [absorbanceat] absorbance at 260 nm.

Paragraph at page 18, fourth paragraph, lines 21-26:

50 µl of each of the ten PCR were mixed and loaded on a 1% TBE agarose gel. After migration and staining with ethidium bromide, the band at 2572 bp, corresponding to the amplification product of the genes of the ten mutants, was cut out with a scalpel and purified with the [Quiaquick] QIAquick system (QIAGEN). [Allthe] All the DNA thus purified was eluted in 120

µl of buffer T. The concentration of this DNA was approximately 100 ng/µl according to its absorbance at 260 nm.

Paragraph at page 21, 2nd paragraph, lines 8-12:

The PCR amplification products of the RLR 1, 2 and 3 reactions were purified with the [Wizard PCR Preps] WIZARD PCR PREPS system (PROMEGA) and eluted in 45 µl of buffer T. 6 µl of each purified PCR were incubated 1 hour at 37 °C in a mixture containing 3 µl of restriction buffer C, 3 µl of BSA (1 mg/ml), 20 U of the *Eco* RI enzyme, 10 U of the *Nco* I enzyme and 15 µl of water.

Paragraph at page 21, fourth paragraph, lines 20-23:

The linearized vectors as well as the digested PCR were purified on a TBE 1% agarose gel with the QIAquick system [(QUIAGEN)] (QIAGEN). Each vector or each digested PCR was eluted in 30 µl of buffer T.